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COMPLETE SPECIFICATION

(54) USE OF LONIDAMINE IN CANCER

PATENT APPLICATION BY: (71)
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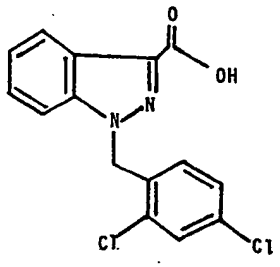
The present invention concerns a new application of a known drug with the generic name lonidamine, formerly referred to as diclondazolic acid. Lonidamine, is 1-(2,4-di-chlorobenzyl)-1H-indazole-carboxylic acid. This compound has the chemical formula:



Lonidamine is the subject of U.S. Pat. No. 3,895,026 which comprises lonidamine itself and various substituted 1-benzyl-1H-indazole-3-carboxylic acids and derivatives; in this patent the drug's pharmacological and therapeutic properties are attributed to its antispermatogenic properties. In this patent mention is also made of a potential use in females to inhibit ovulation or to cure sterility by the rebound mechanism following inhibition of ovulation. Moreover, it is mentioned that lonidamine and its analogues inhibit coagulation of serum proteins in vitro and may be administered in humans for the purpose of treating various inflammatory and degenerative diseases.

The application priority data of the above mentioned U.S. Patent is February 29, 1972, corresponding to the Italian Patent Application N. 48628A/72.

In the Italian Patent application a potential use of lonidamine and its analogues in the therapy of some testicular tumors and prostatic tumors is also mentioned. These uses were cancelled in the patents subsequently applied for in the U.S. because they were not corroborated by any experimental result.

PRIOR ART

The new therapeutic application of lonidamine which is the subject of the present invention concerns the anticancer activity of lonidamine. In this connection, it should be mentioned that a potential interest of lonidamine in the therapy of cancer was speculated upon in the above mentioned Italian Patent application: however, it was restricted to tumors of the testis and prostate and was considered to be the consequence of the antispermatogenic activity of this class of compounds; moreover, this potential use was hypothesized on the basis of theoretical considerations rather than experimental results.

Later on several experimental investigations were conducted to check the possibility that lonidamine possesses an antitumor activity. First of all, lonidamine has been studied in the battery of animal tumors which is currently used for the screening of anticancer agents. The tests were performed according to the procedures outlined in the NCI Protocols for Screening of Anti-Cancer Compounds (Geran et al., 1972).

Animals of both sexes were used; since no difference

was observed between male and female animals, the results were pooled. The most significant points of these protocols may be described as follows:

The P-388 leukemic cells were propagated in the ascitic form using DBA/2 mice. Tumor cells were adjusted to 10^6 cells and implanted intraperitoneally. Mice were randomized by cages. Treatment was given orally or intraperitoneally once a day for 9 days. Data were calculated as median survival time. Mice were observed for death daily until all were dead or up to day 30. The compound was suspended in 0.3% hydroxypropyl cellulose and the concentration was adjusted in order to give a volume of 10 ml/kg of body weight.

The L-1210 leukemic cells were propagated in the ascitic form using DBA/2 and CDF_1 mice. Tumor cells were adjusted to 10^5 cells and implanted intraperitoneally. The tests were performed as in the P-388 experiment.

The melanotic melanoma B-16 was propagated in the form of tumor homogenate (1 g of tumor with 10 ml of BSS) in DBF_1 mice. The tumor homogenate was injected at a volume of 0.5 ml intraperitoneally. The test was performed as described above.

Ependymoblastoma involves a mutant subline of the original methylcholanthrene-induced tumor. A 1X1 mm tumor fragment was implanted intracranially in $B_6C_3F_1$ mice with a trocar. Treatment was performed for 5 days. The test was performed as described above.

The Lewis Lung tumor was propagated by subcutaneous

implant of a 2X4 mm tumor fragment in the axillary region. BDF_1 mice were used. Treatment was performed daily for 9 days by oral gavage, intraperitoneally or in the form of medicated diet. In the latter case, the animals were housed individually and food consumption was determined daily. The test was performed as described above.

The Ehrlich ascites was studied in CF_1 mice. Tumor cells were adjusted to $6x10^6$ and implanted intraperitoneally. Treatment was given orally once a day for 9 or 15 days, or in the form of medicated diet, as described for the Lewis Lung tumor. The animals were observed for 3 months or until death. The test was performed as described above.

The Sarcoma 180 was propagated in the ascitic form in CF_1 mice. Tumor cells were adjusted to $6x10^6$ and implanted intraperitoneally. Experiments were also performed with the solid form of Sarcoma 180. A fragment of 40 mg was implanted subcutaneously in the animals' back. The test was performed as described above.

All these tests were negative except for the Lewis Lung and Sarcoma 180 tumors. Table I summarizes the results of experiments conducted with the Lewis Lung tumor. Antitumor activity was suggested in the experiments in which treatment was given intraperitoneally. In the first experiment performed with this tumor an increased life span of 32 per cent was observed in the group treated with 50 mg/kg i.p. of lonidamine. In the second experiment the increase of life span was 92 per cent at the dose of 100 mg/kg i.p. Both administrations by gavage and medicated diet gave negative

results.

Table II summarizes the results obtained in the Sarcoma 180 ascitic tumor. A 22 per cent increase of life span was observed with 25 mg/kg p.o. of lonidamine, but not with the higher doses. Administration of the drug in the diet resulted in a dose-related increase in life span. Using the Sarcoma 180 in the solid form the efficacy of lonidamine was confirmed. These results were presented the first time on the occasion of a symposium on lonidamine held at L'Aquila in 1979 and will be published in the near future as Proceedings of the above mentioned symposium (Silvestrini, 1981 in press).

Unfortunately the presently available antitumor agents have a wider spectrum of activity in these experimental tumors; on the other hand, different studies conducted in the past three decades have shown that compounds devoid of anticancer activity in humans may occasionally be effective in one or two of the animal models used for the laboratory screening of anticancer drugs (Gellhorn and Hirschberg, 1955; Goldin et al., 1966; Wood, 1977. Consequently, the fact that lonidamine was active in 2 out of 7 animal models did not seem to justify a clinical trial on this drug.

A biochemical investigation of the mechanism of action of lonidamine has provided a different approach to the study of the anticancer activity of lonidamine. Results of these investigations have been reviewed and presented in occasion of the above mentioned symposium (Silvestrini, 1981 in press). The basic idea of these investigations was that

lonidamine affects specifically an energy mechanism used by biological systems, such as germ cells and tumors, which are characterized by high energy requirements at a low oxygen tension. This energy mechanism would correspond, according to the Hackenbrock definition, to condensed mitochondria which have been described both in germ and tumor cells (Hackenbrock, 1971). This working hypothesis has been corroborated by experimental results. Biochemical and ultrastructural studies have shown that lonidamine inhibits respiration of cancer cells and produces morphological changes of their mitochondria; moreover it has been observed that lonidamine inhibits glycolysis of cancer cells as well as respiration (Caputo et al., 1979; Paggi et al., 1979; Floridi et al., 1981; Floridi et al., 1981 in press). It should be recalled that respiration and glycolysis represent two alternative energy systems of living cells: when one is inhibited, the other one is increased and vice versa. Consequently, the fact that lonidamine inhibited in cancer cells both respiration and glycolysis suggested a specific activity on cancer cells. Unfortunately no correlation has been found between the biochemical and ultrastructural effects of lonidamine on cancer cells and in its ability to increase the survival of tumor-bearing animals. In fact, the above mentioned biochemical and ultrastructural observation had been made testing in vitro lonidamine against Ehrlich ascite cells, whereas no increase of survival was found when the drug was administered to animals implanted with this

tumor; in this connection, see the results quoted above (Silvestrini, 1981 in press).

The value of experimental studies conducted on the anticancer activity of lonidamine should be considered with caution. The transplantable tumors currently used in the screening of the anticancer agents present sharp differences with human pathology: the latter involves a transformation of pre-existing cells, whereas the animal models consist only in the transplantation of cancer cells. These animal models are of pratical value to study anticancer agents which affect the mitosis, but there is no proof that they may detect the activity of anticancer agents acting by different mechanisms. Since lonidamine possesses a biochemical mechanism of action entirely different from that of known anticancer agents, its potential value in anticancer therapy could be assessed not on the basis of animal studies, but only on the basis of clinical trials.

CLINICAL STUDIES

The clinical studies were preformed on 10 patients with different types of tumour.

For ethical reasons, the patients chosen were mainly those with histologically proven neoplasia in the advanced stage (stage IV according to the I.U.A.C.) not undergoing radiotherapy or chemo-hormonal therapy for at least 6 weeks. The "performance" state according to Karnofsky (1967) was between 70 and 40. Patients' approval was obtained before admission to the study.

The neoplastic lesions were all measurable and/or assessable clinically and instrumentally. Assessments made by radiography, scanning, and ecography depending on the type of primary tumour and site of the metatastic lesions, were performed at the basal time and every 4 weeks during the treatment period.

Drug tolerance was evaluated by hematology, blood chemistry and function tests, in addition to any clinically detectable side-effects.

Lonidamine was administered orally in the form of 150 mg tablets at the daily dose of 450 mg (1 tablet 3 times daily). This dose was increased after a month to 900 mg (2 tablets 3 times daily) when the therapeutic response was absent and tolerance good. Experimental treatment lasted 12 weeks. During this period no other antitumoural drug was given to the patients.

The response to treatment with lonidamine was evaluated according to the following international criteria (Miller

et al., 1981).

Complete response (C.R.)

Partial response (P.R.)

Stationary (ST)

Progress (PROG)

Table III reports the characteristics of the patients admitted to the study and the results obtained.

The results obtained at the end of 12 weeks treatment may be summarized as follows: 1 complete response, 5 partial responses, 1 stationary and 3 progress.

Complete remission occurred in a patient with a single cerebral metatastasis of primary carcinoma of the breast which had been surgically removed. The cerebral metastasis involving the right temporal region, visible and measurable on cerebral tomography (CAT) performed at basal time was no longer detectable on weeks 8 and 12. Partial remission involved 5 cases: 1 adenocarcinoma of the ascending colon, 1 adenocarcinoma of the prostate, 2 carcinoma of the lung, 1 breast carcinoma. Partial remission consisted in a 50% reduction of the sum of the areas of neoplastic lesions accompanied by improvement of clinical symptoms and signs.

Tolerance of the drug on the basis of hematology, blood chemistry and function test was good in all cases: the most common side-effects consisted in myalgia involving the limbs (2 cases), gastralgia and pyrosis (2 cases) and testicular pain (1 case) when the dose was increased on week 5 of treatment.

TABLE I
Effects of lonidamine on Lewis Lung tumour in the mouse[a]

Daily dose (mg/kg) and route	MST[b]	T/C%[c]	ILS%[d]
0	19.0		
50 p.o.	19.0	100	
100 p.o.	16.3	86	
200 p.o.	20.0	105	5
400 p.o.	7.0	41	
0	16.1		
50 i.p.	21.3	132	32
100 i.p.	19.0	118	18
200 i.p.	toxic		
0	17.3		
50 i.p.	33.3	192	92
100 i.p.	35.8	206	106
0	32.0		
100 medic.diet[e]	31.3	97	

(a) 10 mice were used for each dose

(b) Median Survival Time

(c) MST of test group/MST of 0 dose group x 100

(d) Increased Life Span

(e) Concentration of lonidamine 0.08%

TABLE II
Effects of lonidamine on ascitic Sarcoma 180 in the mouse

Daily dose (mg/kg)and route	No. of mice	Days of treatment	MST[a] (day)	T/C%[b]	ILS%[c]
0	19	1-9	22.4		
25 p.o.	20	1-9	27.4	122	22
50 p.o.	15	1-9	22.0	98	
100 p.o.	19	1-9	24.4	109	9
0	20		20.3		
62 medic. diet (e)	19	0-until death	21.1	104	4
0	37		21.2		
125 medic. diet (f)	37	0-until death	26.3	124	24
0	30		23.3		
250 medic. diet (g)	29	0-until death	35.3	151	51
0	21		54.5[d]		
125 medic. diet (f)	19	0-until death	72.5[d]	133	33

(a) Median Survival Time
(b) MST of test group/MST of 0 dose group x 100
(c) Increased Life Span
(d) Sarcoma 180 in the solid form
(e) Concentration of 0.04%
(f) Concentration of 0.08%
(g) Concentration of 0.17%

TABLE III

Clinical study of the antitumoral activity of lonidamine

Pat. No.	Name	Sex	Age (years)	Height cm	Weight kg	Diagnosis
1	V.G.	M	55	1.66	62	Adenocarcinoma of the colon
2	E.B.	M	58	1.72	60	Adenocarcinoma of the prostate
3	G.C.	F	40	1.68	87	Infiltrating duct carcinoma of the breast
4	A.C.	F	52	1.70	61	Infiltrating duct carcinoma of the breast
5	A.S.	F	60	1.52	48	Tubular adeno-carcinoma of the breast

TABLE III (Cont.)

Clinical study of the antitumoral activity of lonidamine

Pat. No.	Site of metastasis	Stage	Performance status	Previous treatment	Response to lonidamine
1	lymph node liver	IV	50	Surgery, radio-therapy, chemo-therapy	P.R.
2	lymph node lung	IV	60	Surgery, chemo-hormonal therapy	P.R.
3	bone	IV	70	Surgery, chemo-hormonal therapy	ST
4	bone lung	IV	60	Surgery, radio-therapy, chemo-hormonal therapy	PROG
5	bone brain	IV	40	Surgery, radio-therapy, chemo-hormonal therapy	P.R.

TABLE III (Cont.) - Clinical study of the antitumoral activity of lonidamine

Pat. No.	Name	Sex	Age (years)	Height cm	Weight kg	Diagnosis
6	S.M.	F	64	1.58	49	Infiltrating duct carcinoma of the breast
7	F.L.	F	61	1.50	42	Infiltrating duct carcinoma of the breast
8	M.E.	M	52	1.64	68	Small cell carcinoma of the lung
9	G.M.	F	56	1.67	67	Epidermodal carcinoma of the lung
10	O.P.	M	49	1.57	50	Epidermodal carcinoma of the lung

TABLE III (Cont.) - Clinical study of the antitumoral activity of lonidamine

Pat. No.	Site of metastasis	Stage	Performance status	Previous treatment	Response to lonidamine
6	brain	IV	40	Surgery, chemo-therapy	C.R.
7	bone, liver brain	IV	40	=	PROG
8	brain	IV	40	=	P.R.
9	brain	IV	40	=	P.R.
10	brain, bone	IV	40	=	PROG

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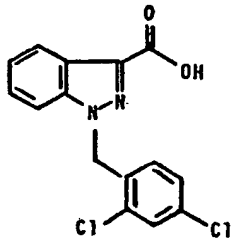
CLAIMS

1. Use of lonidamine of the formula



in the manufacture of a pharmaceutical formulation for the treatment of cancer.

2. Use as claimed in claim 1 wherein the lonidamine formulation is in a form suitable for oral administration.

3. Use as claimed in claim 1 or 2 wherein the lonidamine is administered in a amount of between 450 and 900 mg per day.

4. Use of lonidamine in the manufacture of a pharmaceutical formulation for the treatment of cancer substantially as hereinbefore described with reference to the Examples.

Dated this 30 day of June 1982,
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